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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Large quantities of parasite materials(*S mansoni)* were supplied to investigators at the National Naval Medical Research Institute in Bethesda, Maryland for immunoparasitological research. Research on host-parasite relationships designed (1) to weight the balance in favor of the schistosome and so increase the production of available material, and (2) to clarify the invasive mechanisms resulted in the following findings. There were differences in cercarial production in intraspecific *Schistosoma mansoni-Biomphalaria glabrata* strain models. In the model in use, variability was encountered in cercarial output, miracidial infectivity, and snail death rates. Causes of some

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Block 20. Abstract Continued. of this variability were identified. The limits of variability under our conditions were specified. An optimal maintenance system for this schistosome-snail strain model was developed. Rotifer inhibition of cercarial output and motility was discovered. A jet spray wash control procedure for rotifers was designed.

OFFICE OF NAVAL RESEARCH

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(6) Schistosome Materials for Vaccine Development,

by

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(10) M. Stirewalt and F. Lewis

Biomedical Research Institute
Rockville, Maryland 20852

(11) September 1981

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SUMMARY

This contract has been funded through the Office of Naval Research by the Naval Medical Research Institute (NMRI). It has had 2 purposes: (1) to supply investigators in schistosome immunology at NMRI with parasite and infected-host materials for their research; and (2), researchwise, to define the snail-schistosome-mouse interactions involved in maintenance of a constant, dependable, large-volume source of parasite and infected host materials.

From the beginning, *Biomphalaria glabrata* of mixed Puerto Rican-Brasilian origin (Nmri strain) have been grown and infected each week in sufficient numbers to supply 4 million or more cercariae of Puerto Rican *Schistosoma mansoni* (Nmri strain) weekly. Albino mice have been infected, held for 7 or 8 weeks, sacrificed and perfused, and their adult schistosomes recovered and cleaned for use in antigen preparations. In addition, schistosome eggs, schistosomules, miracidia, infected snails, infected mice and mouse tissues, and cercarial penetration enzymes have been available upon request. (Annual reports No. 1 through 5).

Research has been focussed on three areas. The first was on defining the means of schistosome maintenance at a level which insured a constant dependable supply of large quantities of research material. To this end, maintenance techniques and the harvests of parasite materials have been monitored constantly while one procedure at a time was changed in an effort to understand its effect on parasite production. These techniques included: number of miracidia to which snails were exposed — 1, 5, 6 to 8, and 8 to 10 (ms prepared for submission for publication); temperature and light changes; size of snails at exposure — 4 to 6 mm diameter optimal; and suitability of mouse strains for provision of miracidia (in progress). (Annual reports No. 1 to 5; Stirewalt, M. in press. *Schistosoma mansoni*: Conditions contributing to maximal cercarial harvests. Journal of Parasitology).

The second area of research interest was in development of procedures for collecting penetration enzymes of cercariae and analyzing its total protein content (Lowry) and its activity against an azocoll substrate. Total protein content varied widely from collection to collection but the proteolytic activity remained fairly constant. (Annual Report No. 1; Campbell, D. L., Frappaolo, P. J. E., Stirewalt, M. A. and Dresden, M. H. 1976. *Schistosoma mansoni*: partial characterization of enzyme(s) secreted from the preacetabular glands of cercariae. Experimental Parasitology *39*, 33-40). Immunogenicity of the enzymes was demonstrated by induction in mice of precipitating and reaginic antibodies, but no protection was afforded mice against a challenge infection. (Annual report No. 1; Minard, P., Murrell, K. D., and Stirewalt, M. A. 1977. Proteolytic, antigenic and immunogenic properties of *Schistosoma mansoni* cercarial secretion material. American Journal of Tropical Medicine and Hygiene *26*, 491-199). This work was supported also by N00014-76-0053.

Research of the third type resulted in the finding that certain rotifers which colonized the shells of infected snails strongly inhibited cercarial emergence. (Annual report No. 2; Stirewalt, M. and Lewis, F. A. 1981. *Schistosoma mansoni*: effect of rotifers on cercarial output, motility and infectivity. International Journal for Parasitology *11*, 301-308). Methods of controlling rotifer colonization on snails were developed. They consist of treating each snail with 10% ethanol applied to the shell by swab; or washing the rotifers from snails individually by a jet stream of water. (Annual report No. 4).

Drastic reduction in support for this contract has eliminated funding for research since FY78.

INDEX OF ANNUAL REPORTS

1. Annual report, 15 July, 1976
2. Annual report, 30 September, 1977
3. Annual report, 21 September, 1978
4. Annual report, 24 September, 1979
5. Annual report, 24 September, 1980

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CONCLUSIONS

1. It is possible to produce in quantity parasite materials for research on *Schistosoma mansoni*. Our production has been as follows: 1 to 2 million cercariae per day; 30,000 adult worms per week; 200,000 schistosomules per day; other materials as needed.
2. Requirements for such production are: money, time, space, personnel and constant monitoring.
3. Intraspecific strains of the *Biomphalaria glabrata* and *Schistosoma mansoni* in the model association vary in productivity level. A suitable model must be set up.
4. Base line parameters and standards were: 2500 or more cercariae/snail/day; 100 or more adult worms/mouse; snail infection rates of 90% or more; death rates of infected snails 10% or less biweekly.
5. Exposure level of snails need not exceed 6 to 8 miracidia/snail, since only 2 or 3 primary sporocysts appear to develop concurrently in a snail.
6. Temperature and light changes stimulate cercarial emergence. Shedding snails should be maintained in the dark at about 27 C and placed in strong light at about 32 C for cercarial collection.
7. Shedding snails must be kept free of colonizing rotifers.
8. Proteolytic penetration enzymes can be collected from cercariae by holding them at 37 C over a substrate of skin surface lipid or its active ingredients, linolenic or linoleic acids.
9. These secreted enzymes are antigenic, but the antibodies which are stimulated are not protective.

MAJOR ACCOMPLISHMENTS

1. Maintenance of an intraspecific *Schistosoma mansoni-Biomphalaria glabrata* strain model which produces a constant high level of research material.
2. Recorded cercarial output, miracidial infectivity and snail death rates from 1976 to present as base line information for our snail-schistosome model.
3. Development of the optimal cercarial production system which relies on (a) 2 groups of infected snails from each of which cercariae are harvested twice weekly; (b) design of the 27 C dark maintenance and 32 C lighted cercarial harvest conditions.
4. Report of differences in cercarial productivity by two intraspecific strains of the *Schistosoma mansoni-Biomphalaria glabrata* model.
5. Discovery that colonization of infected snail shells with rotifers decreases cercarial output and inhibits cercarial motility.
6. Design of jet spray washing of rotifers from snails.

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